Severe Immunodeficiency Has Opposite Effects on Neuronal Survival in Glutamate-Susceptible and -Resistant Mice: Adverse Effect of B Cells[1]

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The resistance of rats or mice to glutamate-induced toxicity depends on their ability to spontaneously manifest a T cell-dependent response to the insult. Survival of retinal ganglion cells (RGCs) exposed to glutamate in BALB/c SCID mice (a strain relatively resistant to glutamate toxicity) was significantly worse than in the wild type. In the susceptible C57BL/6J mouse strain, however, significantly more RGCs survived among SCID mutants than in the matched wild type. RGC survival in the SCID mutants of the two strains was similar. These results suggest 1) that immunodeficiency might be an advantage in strains incapable of spontaneously manifesting protective T cell-dependent immunity and 2) that B cells might be destructive in such cases. After exposure of RGCs to toxic glutamate concentrations in three variants of B cell-deficient C57BL/6J mice, namely muMT$^{-/-}$ (B cell knockout mice) and Ii$^{-/-}$ mice reconstituted with transgenically expressed low levels of Ii p31 isoforms (p31 mice) or Ii p41 isoforms (p41 mice), significantly more RGCs survived in these mice than in the wild type. The improved survival was diminished by replenishment of the B cell-deficient mice with B cells derived from the wild type. It thus seems that B cells have an adverse effect on neuronal recovery after injury, at least in a strain that is unable to spontaneously manifest a T cell-dependent protective mechanism. These findings have clear implications for the design of immune-based therapies for CNS injury. *The Journal of Immunology,* **2002, 169: 2861–2865.**

The cross-talk between the nervous system and the adaptive arm of the immune system after CNS trauma has only recently attracted the attention of scientists (1–3). Immune components (such as T and B cells) that participate in adaptive immune responses were not considered to be important players in the aftermath of CNS insults. Studies in our laboratory have shown, however, that axonal injury in the CNS evokes a T cell-mediated protective immunity that can be boosted by passive or active vaccination with myelin-associated Ags (4–6). They further showed that not all individuals are capable of spontaneously manifesting this protective immunity, although all can benefit from its boosting (7, 8). The ability to spontaneously exhibit protective immunity was correlated with resistance to the development of the autoimmune syndrome experimental autoimmune encephalomyelitis (EAE).[4] Those studies were conducted in various mice and rat strains, including congenic mice such as C3H/SW and C3H/Hej, which differ only in their H-2 haplotype (9, 10).

Subsequent studies by our group showed that T cells are also beneficial in cases of stress caused by a toxic excess of physiological compounds such as glutamate. In individuals capable of spontaneously exhibiting a T cell-mediated protective immunity (i.e., resistant strains), the number of neurons that survive the glutamate insult is significantly greater than in individuals lacking this ability. Moreover, after intravitreal injection of toxic amounts of glutamate, nude mice (i.e., mice devoid of mature T cells) of strains in which the wild type is resistant lose significantly more retinal ganglion cells (RGCs) than the wild type. Furthermore, in susceptible strains (i.e., those with only a limited ability to manifest a T cell-dependent protective immunity in response to glutamate insult), not only is neuronal survival after CNS injury significantly lower than in resistant strains but also the absence of mature T cells does not affect the outcome of the insult (9, 11).

In view of the above findings, we were interested in investigating the part played by B cells in the immune response to CNS insults. In the present study, we measured RGC survival in SCID mice of both resistant and susceptible strains after intravitreal exposure to glutamate toxicity. These mice are devoid of both mature T cells and B cells. We found that immunodeficiency had opposite effects in each of the two strains, leading us to postulate that B cells have an adverse effect on neuronal survival in susceptible strains.

We examined this working hypothesis in a susceptible mouse strain (C57BL/6J), using the wild type and three different types of mutants deficient in their mature B cell populations: muMT$^{-/-}$ mice and p31 and p41 transgenic mice (Ii$^{-/-}$ mice that were reconstituted with transgenically expressed low levels of the Ii p31 or p41 isoform (p31 or p41 mice, respectively) (12). These transgenic mice have normal Ag presentation and T cell populations but a defect in their B cell differentiation (13, 14). Our results suggest that, at least in strains with a poor ability to regulate their autoimmune T cell response (i.e., susceptible strains), B cells have a negative effect on recovery from CNS injury.

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[4] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; RGC, retinal ganglion cell.

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Materials and Methods

Animals

The mice used in this study were handled according to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. Male wild-type and SCID mice of the C57BL/6J and BALB/c strains were used. We also used three types of B cell-deficient C57BL/6J mice, namely, p31, p41 (both of these are $Ii^{-/-}$ mice reconstituted with Ii p31 and p41 isoforms, respectively) (12), and $muMT^{-/-}$ mice (The Jackson Laboratory, Bar Harbor, ME). The mice were between 8 and 13 wk of age. Mice of each of the above types were anesthetized by i.p. administration of ketamine (80 mg/kg) and xylazine (16 mg/kg). Before tissue excision, the mice were killed with a lethal dose of pentobarbitone (170 mg/kg).

Labeling of RGCs

RGCs were labeled 72 h before tissue excision with a fluorescent dye injected stereotactically into the superior colliculus. For this purpose, mice were anesthetized and placed in a stereotactic device. The skull was exposed and kept dry and clean, and the bregma was identified and marked. The designated point of injection was 2.92 mm posterior to the bregma, 0.5 mm lateral to the midline, and at a depth of 2 mm from the brain surface. A window was drilled in the scalp above the designated coordinates in the right and left hemispheres. The neurotracer dye FluoroGold (5% solution in saline; Fluorochrome, Denver, CO) was applied (1 μl, at a rate of 0.5 μl/min in each hemisphere) using a Hamilton syringe, and the skin over the wound was sutured.

Glutamate injection

With the aid of a binocular microscope, the right eye of the anesthetized mouse was punctured in the upper part of the sclera with a 27-gauge needle, and a 10-μl Hamilton syringe with a 30-gauge needle was inserted as far as the vitreal body. Mice were injected with a total volume of 1 μl of L-glutamate (Sigma-Aldrich, St. Louis, MO), dissolved in saline.

Assessment of RGC survival

At the end of the experimental period, the mice were given a lethal dose of pentobarbitone (170 mg/kg). Their eyes were enucleated and the retinas were detached and prepared as flattened whole mounts in 4% paraformaldehyde in PBS. Labeled cells from four to six fields of identical size (0.076 mm^2) were counted. The counted fields were located at approximately the same distance from the optic disk (0.3 mm) to allow for variations in RGC density as a function of distance from the optic disk. Fields were counted under the fluorescence microscope (magnification, ×800) by observers blinded to the treatment received by the mice. The average number of RGCs per field was calculated for each retina. The number of RGCs in the contralateral (uninjured) eye was also counted and served as an internal control.

Isolation of B cells

Spleen cells were obtained from C57BL/6J (wild-type) mice ages 6–8 wk. The B cell population was enriched by treatment of the splenocyte suspension with Abs against surface molecules known to exist on T cells (anti-Thy-1 (CD90), CD4, and CD8; Southern Biotechnology Associates, Birmingham, AL) for 1 h, followed by incubation with Low Tox-M complement (Cedarlane, Hornby, Canada) for 1 h at 37°C. Purified B cells were injected i.v. into p31 transgenic mice (4×10^7 B cells per mouse).

Results

Immunodeficiency has opposite effects on neuronal survival in mice susceptible and resistant to glutamate toxicity

We first compared RGC survival in SCID and wild-type mice of two strains, BALB/c and C57BL/6J. The disparity in RGC survival between SCID and wild-type mice varied in the two strains (Fig. 1). In BALB/c mice (a glutamate-resistant strain capable of spontaneously manifesting a T cell-mediated protective immunity (9)), RGC survival (expressed as the mean number of RGCs per square millimeter ± SEM) was worse in the SCID mutants (1687 ± 71) than in the wild type (2122 ± 42; $p < 0.001$). In contrast, RGC survival in SCID mutants of the susceptible C57BL/6J (9) strain was better than in their matched wild-type controls (1665 ± 140 compared with 980 ± 85; $p < 0.006$). Interestingly, RGC outcome

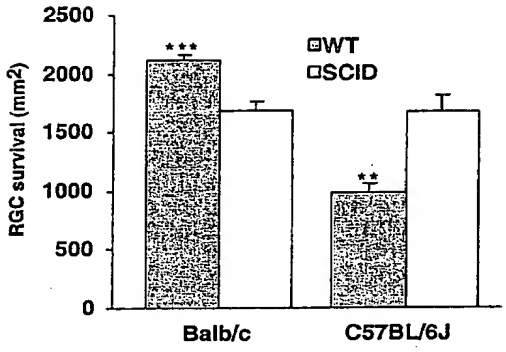

FIGURE 1. The outcome of exposure to glutamate toxicity in SCID mice is strain dependent. C57BL/6J and BALB/c SCID and wild-type mice were given a single intravitreal injection of 200 nmol glutamate (n = 10–12 in each group). Surviving RGCs were counted 7 days later. RGC survival is expressed as the mean number of RGCs ± SEM per square millimeter. **, $p < 0.05$; ***, $p < 0.0005$ (Student's t test). Number of RGCs in normal eye of WT BALB/c, WT C57BL/6J, SCID BALB/c, SCID C57BL/6J are 3100 ± 44, 3220 ± 38, 3304 ± 140, 3162 ± 90, respectively.

in the SCID mice of the two strains was similar. It thus appears that RGC survival is maximal in the presence of an endogenous T cell-dependent protective mechanism. SCID worsens the neuronal outcome in resistant strains; however, this outcome is still better than that in the wild-type susceptible strain. These findings point to the possible presence of a destructive mechanism in susceptible strains (Fig. 1).

B cells have an adverse effect on neuronal survival in susceptible mice

With the use of the glutamate-susceptible C57BL/6J strain, studies in our laboratory have shown that neuronal survival after exposure to glutamate toxicity in nude mice and in their matched wild-type controls is similar (11). This finding raised the question of whether the effect of B cells in this strain is destructive. To examine how B cells affect RGC survival after exposure to glutamate toxicity, we examined the $muMT^{-/-}$ mutant (B cell knockout) of the C57BL/6J mouse strain (Fig. 2). The number of neurons (mean ± SEM) that survived glutamate toxicity was significantly higher ($p < 0.0005$; two-tailed t test) in the $muMT^{-/-}$ variant (1835 ± 76) than in the wild-type control (1387 ± 57) (Fig. 2). We further tested two additional mouse variants defective in B cell maturation

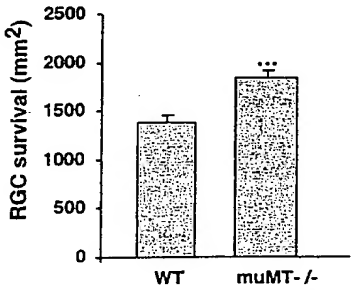

FIGURE 2. The $muMT^{-/-}$ (B cell knockout) mutant of C57BL/6J mice is more resistant than the wild-type to glutamate toxicity. Wild-type and muMT C57BL/6J mice were each given a single intravitreal injection of 200 nmol glutamate. Surviving RGCs were counted 7 days later. RGC survival is expressed as the mean number of RGCs ± SEM per square millimeter. ***, $p < 0.0005$ (Student's t test). Number of RGCs in normal $muMT^{-/-}$ mice is 3500 ± 165.

(p31 and p41, both on a background of the susceptible strain C57BL/6J). In both cases (p31, 1825 ± 96; p41, 1822 ± 92), recovery from glutamate toxicity was significantly better ($p < 0.01$ in both cases) than in the wild type (1378 ± 121) (Fig. 3). Moreover, the numbers of RGCs in the uninjured eyes were similar in all of the tested mouse strains (C57BL/6J, 3220 ± 38; p41, 3186 ± 69; p31, 3095 ± 89; muMT$^{-/-}$, 3100 ± 56). The same pattern was observed when we repeated the comparison in the p41 mutant after optic nerve crush (data not shown). Fig. 4 shows representative photographs of the retinas excised from p31 mice or from wild-type C57BL/6J mice after their exposure to glutamate. As control, the uninjured eye is shown.

B cells from wild-type mice reduce neuronal survival in B cell-deficient mice

To confirm the correlation between improved survival and absence of mature B cells, we replenished the p31 mutant of the C57BL/6J mice with B cells obtained from the wild type. In the replenished mice, the numbers of surviving RGCs dropped from 1825 ± 96 to 1480 ± 119 ($p < 0.04$, p31 vs p31 replenished with B cells; $p < 0.02$, p31 vs wild type) (Fig. 5). Thus, transfer of mature B cells from the matched wild-type control to B cell-deficient mice abolished the survival advantage of the B cell-deficient mice. The B cell preparation was tested for purity by FACS using B220 as the B cell marker and was found to be ~85%.

Discussion

The results of this study show that after exposure to glutamate toxicity, the absence of mature B cells in mice devoid of appropriately regulated T cells is advantageous for recovery. Similar results were observed following optic nerve injury (data not shown). In other words, mice deficient in both T cells and B cells may have an advantage in certain cases of malfunctioning immunity. However, in strains that are capable of spontaneously manifesting a beneficial immunity (e.g., EAE-resistant strains (9)), the lack of T cells and B cells is a disadvantage in the event of CNS insult.

B cells are known to display a variety of characteristics other than Ab production. They participate in the development of the lymphoid architecture and contribute to the regulation of T cell subsets and dendritic cell function via the production of cytokines and activation of T cells (16, 17). Little information is available, however, on the infiltration of B cells into injured neuronal tissues or on the role of peripheral B cells in spontaneous recovery from CNS injuries. This study demonstrates for the first time that B cells have a negative effect on neuronal survival, at least in mouse strains with a limited ability to manifest a protective T cell-mediated response to CNS injury (and that are also susceptible to autoimmune disease development). A recent study of the possible accessibility of B cells to the damaged optic nerve in rats showed that B cells accumulate at the lesion site after injury (18).

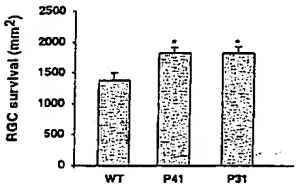

FIGURE 3. The p41 and p31 variants of C57BL/6J mice are more resistant than the wild-type mice to glutamate toxicity. Wild-type C57BL/6J mice and their p41 and p31 transgenic mice were each given a single intravitreal injection of 200 nmol glutamate. Surviving RGCs were counted 7 days later. RGC survival is expressed as the mean number of RGCs ± SEM per square millimeter. *, $p < 0.05$ (Student's t test). Number of RGCs in normal eye of p41 and p31 mice are 3186 ± 69 and 3095 ± 84, respectively.

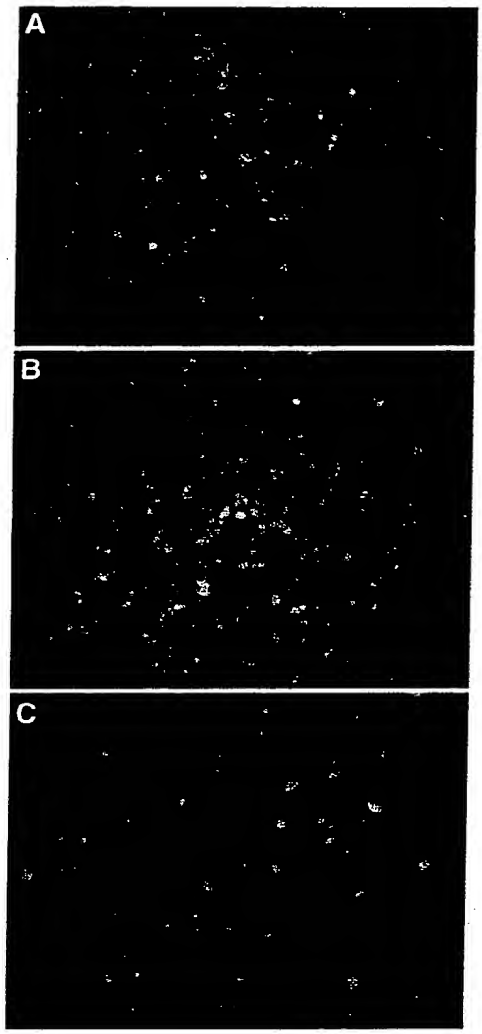

FIGURE 4. Photomicrographs of normal and glutamate-injected mouse retina (C57BL/6J or P31), both stereotactically labeled with FluoroGold and excised 7 days after the insult. *A*, Normal retina; *B*, p31 injured retina; *C*, wild-type injured retina. The photographs were taken in the fluorescence microscope at a magnification of ×25.

Until recently, participation of the immune system in the events that follow CNS insult was considered to be harmful. Studies in our laboratory have introduced a number of changes in this perception by demonstrating that the immune system (in particular macrophages and T cells) plays a significant role in recovery from CNS insults. The local effect of T cells is presumably exerted on effector cells such as resident microglia or invading macrophages. The possible contribution of B cells, however, has received no research attention. The literature contains some reports of an increase in the amount of Abs specific to neuronal Ags, both at the injury site and systemically, after CNS injury in the rat (19–21).

Our findings in SCID mice indicated that B cells might have an adverse effect on neuronal survival, at least in a mouse strain with a limited ability to spontaneously manifest T cell-mediated protection (e.g., C57BL/6J). Thus, SCID mice of the C57BL/6J strain (susceptible) showed better survival after exposure to glutamate

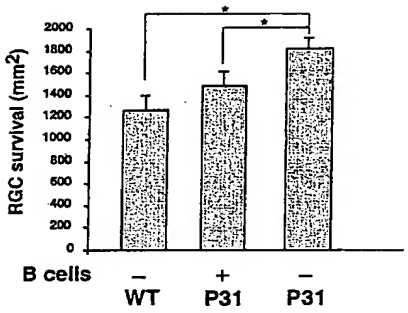

FIGURE 5. Replenishment of C57BL/6J p31 mice with B cells eliminates their relative advantage with respect to neuronal survival. *A*, B cells from wild-type C57BL/6J mice were injected i.v. into C57BL/6J p31 transgenic mice that were previously injected intravitreally with 200 nmol glutamate. RGC survival after 7 days is expressed as the mean number of RGCs ± SEM per square millimeter (n = 5–9 in each group). *, $p < 0.05$ compared with the wild-type control (Student's t test). *B*, Features of the isolated transferred B cells.

toxicity than their wild-type counterparts. Previous studies by our group showed that T cell deprivation in C57BL/6J mice did not affect neuronal survival. Taken together, these findings suggested that in this mouse strain B cells have an adverse effect. C57BL/6J mice that lack mature B cells recovered significantly better from CNS insults than their wild-type counterparts. Furthermore, replenishment of these mature B cell-deficient mice with mature B cells from wild-type C57BL/6J mice diminished this relative advantage.

All $Ii^{-/-}$ mutants of the C57BL/6J strain are similarly deficient in mature B cells, but their T cell responses are normal. These mice, like the wild type, develop EAE and have normal levels of mature MHC-II molecules and $CD4^+$ T cells. (15).

Whether B cells adversely affect CNS recovery in strains capable of spontaneously resisting the consequences of a CNS insult has yet to be discovered. BALB/c mice (glutamate resistant) showed better RGC survival than C57BL/6J mice (glutamate susceptible) in the wild type, in SCID mice (this study), and in nude mice (11). In both strains, survival was better in SCID mice than in the corresponding nude mice. These results suggest that B cells may also adversely affect neuronal survival after injury in the resistant BALB/c mouse strain but that this effect is apparently outweighed by the beneficial effect of the T cells in these mice. Alternatively, they might suggest that the effect of B cells is negative only in nude mice, which lack regulatory T cells, and that in wild-type BALB/c mice, B cells have either no or a positive effect. Resolution of this issue must await the availability of B cell knockout mice of the BALB/c strain or any strain that can spontaneously resist glutamate toxicity.

The present results not only further support our previous contention that T cells are beneficial for neuronal survival but also suggest that their absence or malfunction opens the way for a negative B cell effect. B cells can participate in T cell activation (17), influence the pattern of the immune response through the production of cytokines (22–26), and secrete Abs that are locally produced (within the CNS) (27). The negative influence of B cells on neuronal survival may be a local effect due to their direct interaction with T cells. Alternatively, the local effect might distort the phenotype of the B cells and consequently change the repertoire of their secreted cytokines from one that favors neuronal survival to one that is nonsupportive and even destructive. In line with this possibility is the reported diversity of the cytokine repertoire produced by B cells and its correlation with their phenotype (26).

Recent findings in our laboratory suggest that Th1 is the T cell phenotype that is needed for immune neuroprotection (28). Another recent study showed that B cells can efficiently restimulate only Th2 cells (29). Thus, whereas macrophages or dendritic cells may activate Th1 cells, B cells might interfere with such activation by activating Th2 cells.

It is apparent from the present study and other studies that immune participation in the protection of neuronal tissue from self-destructive compounds is T cell dependent (4–6, 9, 11). It thus seems that in the absence of proper T cell regulation, immunodeficiency is advantageous for neuronal survival. The opposite is true when the appropriate T cells operate. This finding is in line with the exacerbation of facial motoneuron loss after facial nerve transection described in EAE-resistant SCID mice (30, 31). In any event, the proper operation of T cell immunity achieves the best results. The results of this study support the concept of a role of the immune system in defense against self-components and not only against non-self. These results also provide the important lesson that what holds for CNS-immune system interactions in the injured CNS of one strain is not necessarily true for another. Specific immune modulation should be considered in all cases, with the idea of shifting the malfunctioning T cells toward making a positive contribution to recovery, thus securing the maximum benefit that immune defense can provide.

Acknowledgments

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